Review

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Polo-like kinase 1 (PLK1) signaling in cancer and beyond

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List of abbreviations:

AML: acute myeloid leukemia

APC/C anaphase promoting complex/cyclosome

CDK: Cyclin-dependent kinase

DDR: DNA Damage Response

EMT: epithelial mesenchymal transition

HSCs: hepatic stellate cells

MCC: mitotic checkpoint complex

NSCLC: non-small cell lung cancer cells

ORC: origin recognition complex

PBD: Polo-box domain

PCM: pericentriolar matrix

PI3K: phosphatidylinositol 3-kinase

PLK1: Polo-like kinase 1

pre-RC: pre-replicative complex

SAC: spindle assembly checkpoint

γ-TuRC : γ-tubulin ring complex

Abstract

PLK1 is an evolutionary conserved Ser/Thr kinase that is best known for its role in cell cycle regulation and is expressed predominantly during the G2/S and M phase of the cell cycle. PLK1-mediated phosphorylation of specific substrates controls cell entry into mitosis, centrosome maturation, spindle assembly, sister chromatid cohesion and cytokinesis. In addition, a growing body of evidence describes additional roles of PLK1 beyond the cell cycle, more specifically in the DNA damage response, autophagy, apoptosis and cytokine signaling. PLK1 has an indisputable role in cancer as it controls several key transcription factors and promotes cell proliferation, transformation and epithelial-to-mesenchymal transition. Furthermore, deregulation of PLK1 results in chromosome instability and aneuploidy. PLK1 is overexpressed in many cancers, which is associated with poor prognosis, making PLK1 an attractive target for cancer treatment. Additionally, PLK1 is involved in immune and neurological disorders including Graft versus Host Disease, Huntington's disease and Alzheimer's disease. Unfortunately, newly developed small compound PLK1 inhibitors have only had limited success so far, due to low therapeutic response rates and toxicity. In this review we will highlight the current knowledge about the established roles of PLK1 in mitosis regulation and beyond. In addition, we will discuss its tumor promoting but also tumor suppressing capacities, as well as the available PLK1 inhibitors, elaborating on their efficacy and limitations.

1. Introduction

Polo-like kinase 1 (PLK1) is the most extensively studied member of the PLK kinase family, mostly in the context of cell cycle regulation and cancer (1). Genetic ablation or inhibition of PLK1 results in aberrant chromosome segregation and as a result - mitotic block, often accompanied by cell death (2). PLK1 is often overexpressed in a wide range of tumors and is linked to poor clinical outcomes. Furthermore, PLK1 overexpression has been linked with resistance to chemotherapy and PLK1 inhibition enhances sensitivity of cancer cells to chemotherapy and radiation (3,4). Because of these interesting features, research and knowledge on the role of PLK1 in mitosis and tumor promotion have grown extensively over the years, which has been nicely reviewed in several review papers (3,5,6). Nevertheless, new substrates and functions for PLK1 are still being discovered, both in the context of cell cycle regulation and beyond. For instance, recent reports provide additional insights into the role of PLK1 in chromosomal stability, autophagy and DNA damage response, amongst others (7) (8) (9) (10) (11,12). Moreover, a role for PLK1 has been recently described in diverse immune disorders including Graft versus Host Disease, where it contributes to immunological responses of alloreactive T cells (13), or in liver fibrosis (14). Also, in neurological disorders such as Huntington's disease and Alzheimer's disease, activity of PLK1 is altered in affected neurons (15,16). So far, PLK1 is most extensively studied as an attractive target in cancer treatment and several small compound PLK1 inhibitors have been developed over the past years. However, although PLK1 inhibitors show anti-tumor effects in preclinical cancer models and also initially demonstrated promising results in clinical trials (17), they have not achieved a satisfactory therapeutic effect so far, due to dose-limiting toxicities (recent status is discussed below) (3).

In this review we will give an overview and update of the well-established and newly identified regulatory network of PLK1, its extensive role in cell cycle regulation and DNA damage response, as well as its functions beyond the cell cycle. Additionally, we will elaborate on the complex role of PLK1 in cancer and other disorders. We will give an update of existing PLK1 inhibitors in research and their current status in clinical trials, and address the reasons why clinical use of PLK1 inhibitors still comes with limitations and challenges.

2. Structure/function and regulation of PLK1

PLK1 is the most highly conserved polo-like protein among *D. melanogaster* (Polo), *C. elegans* (PLK1), *Xenopus* (Plx1) and mammals (PLK1). In humans five PLK1 paralogues have been identified, known as PLK1, PLK2 (Snk), PLK3 (Fnk/Prk), PLK4 (Sak) and PLK5 (18–22). PLK1 is spatially and temporally enriched at three distinct subcellular locations: the mitotic centrosomes, the kinetochores and the cytokinetic midbody; but it can be also found in both nucleus and cytoplasm during S phase, G2 phase and prophase of mitosis (1). PLK2 and PLK4 are expressed in the G1/S and S phases of the cell cycle, respectively, and are primarily required for centriole duplication and centrosome function in mammalian cells (23,24). PLK3 is required for entry into the S phase of the cell cycle (25) and contributes to DNA damage checkpoint activation (26). Finally, PLK5 does not have a cell cycle role and is rather downregulated in proliferating cells, while it is upregulated in quiescent cells. PLK5 is mainly expressed in brain tissue where it is involved in axonal growth of neuroblasts (27).

The PLK proteins are comprised of two C-terminally located polo-box domains (PBD) and an N-terminal catalytic kinase domain (Figure 1). PLK4 is the most divergent protein of the PLKs, as it contains a cryptic polo-box domain and one PBD, which shares only 16%

homology with the PBDs of the other PLKs (28). On the other hand, PLK5 has lost part of its kinase domain, making it unique in humans, but since it has retained its PBD sequence, it is still considered to belong to the PLK family. The PBD domain plays a key role in the specific subcellular localization of PLK1 by interacting with phosphorylation sites of targeted substrates (29). The optimal binding motif for the PBD is the sequence Ser-(pSer/pThr)-(Pro/X), where X indicates any amino acid except Cys (29). Using affinity purification and mass spectrometry, Lowery et al. identified more than 600 proteins in human osteosarcoma U2OS cells that showed phosphorylation-dependent interaction with PLK1 PBD at different phases of the cell cycle, including proteins in well-studied PLK1-regulated processes but also other proteins involved in translational control, RNA processing and vesicle control (30). A one amino acid change in the Polo box 1 of PLK1 was shown to be sufficient to lead to improper localization of PLK1 and impaired function (31,32). On the other hand, even though the kinase domain is not responsible for the correct localization of PLK1, it most probably aids PLK1 localization to the kinetochores, as a PBD-only PLK1 fragment is mainly localized to the centrosomes and to a lesser extent to the kinetochores (33). Substrate recognition by the PBD not only determines PLK1 localization, but also relieves the auto-inhibitory effect on the N-terminal catalytic domain, resulting in kinase activation for target phosphorylation (34). PLK1 can phosphorylate certain substrates upon their prior phosphorylation by PLK1 itself (self-priming) or rely on the phosphorylation of target proteins by upstream kinases (non-self-priming). For example, PLK1 phosphorylates the centromere scaffold PBIP1 protein at Thr78 allowing the interaction of the PBD of PLK1 (but not of PLK2 or PLK3) with PBIP1 and the timely localization of PLK1 to the kinetochores (35). Another example of PLK1 self-priming is the binding and direct phosphorylation of HsCYK-4 at the spindle midzone, which regulates cytokinesis (36). Alternatively, numerous PLK1

substrates are phosphorylated first by an upstream kinase. WEE-1 kinase, a negative regulator of the G2/M phase, first gets phosphorylated by a cyclin-dependent kinase (CDK), creating a PBD binding motif for PLK1. During mitosis, where PLK1 levels rise, PLK1 binds to WEE-1 at the PBD docking site and phosphorylates it, inducing phosphorylation-dependent degradation of the latter (37). Another example of non-self-priming is BUBR1, which is phosphorylated by CDK1, allowing binding of the PBD and subsequent phosphorylation by PLK1, which ensures stabilization of kinetochore-microtubule interactions during mitosis (38). However, in conditions of high abundance of enzyme and substrate, priming phosphorylation is not absolutely required as studies have shown that PLK1 could phosphorylate substrates *in vitro* in the absence of upstream kinases (29,39–41).

The kinase domain of PLKs is a catalytic T-loop region which allows all the PLK isoforms to exchange ATP for ADP, by transferring the phosphate group to downstream phosphorylation targets (42). The optimal consensus motif for PLK1 phosphorylation is Asp/Glu-X-Ser/Thr-Φ-X-Asp/Glu (X, any amino acid; Φ, a hydrophobic amino acid) (41). Lowery et al. proposed two alternative PLK1-mediated phosphorylation models: processive phosphorylation versus distributive phosphorylation, though these two models are not mutually exclusive (43). During processive phosphorylation, the PBD first binds to a site on a protein that has been previously phosphorylated by a priming kinase, which allows the kinase domain of PLK1 to phosphorylate the same substrate at another site. This implies that PLK1 substrates should contain both PBD-binding sites and kinase phosphorylation motifs. Examples of processive phosphorylation include CDC25C (29,39) , Cyclin B (40) and MYT-1 (41). In this model, PLK1 is spatially and temporally regulated, as PLK1 substrates must be localized in proximity to the PBD, which can also be rapidly dephosphorylated. On the other hand, in the distributive phosphorylation model, the PBD docking to a protein

scaffold allows the kinase domain of PLK1 to phosphorylate a third protein in the complex (or in its proximity). Although no solid example of distributive phosphorylation is known so far, bioinformatics analyses favors the distributive phosphorylation model, as only approximately 1/3 of the eukaryotic phospho-proteomic data were found to both interact with and be phosphorylated by PLK1 (44).

PLK1 activation requires phosphorylation of its kinase domain by other kinases on Ser137 and Thr210 (45,46). Seki et al. showed that two proteins of major importance for PLK1 activation are the kinase Aurora A and its co-factor Bora (47). Mechanistically, CDK1-primed Bora accumulates in the G2 phase and its interaction with PLK1 relieves the auto-inhibition of the PBD, allowing Aurora A to phosphorylate Thr210 on PLK1 (47). This initial activation event takes place outside the nucleus; however, phosphorylation of the PLK1 kinase domain also exposes a nuclear localization signal, allowing the translocation of PLK1 to the nucleus (48). Kasahara et al. also reported phosphatidylinositol 3-kinase (PI3K)-dependent phosphorylation of PLK1 at Ser99, which facilitates its interaction with the 14-3-3γ protein, stimulating PLK1 activity that is required for mitosis progression to anaphase (49). Protein phosphatases further control PLK1 activity during the cell cycle by either dephosphorylating PLK1 itself or PLK1 substrates (50,51). For example, CDK1-primed myosin phosphatase-targeting subunit 1 (MYPT1), a subunit of myosin phosphatase complex, enables protein phosphatase 1 (PP1) to antagonize PLK1 (52,53). Depletion of MYPT1 increases PLK1 phosphorylation on Thr210 (52), while depletion of the B56-PP2A complexes increases the phosphorylation of Aurora B and PLK1 substrates at kinetochores (51).

Ubiquitination is another post-translational modification that contributes to the dynamics of PLK1 activity. In order to balance the increasing expression of PLK1 from G0/G1 to G2/M

phase (54), PLK1 protein levels are reduced in anaphase by ubiquitin-dependent proteolysis initiated by the anaphase-promoting complex/cyclosome (APC/C)^{CDH1} ubiquitin ligase (55). Similarly, reactivation of the APC/C^{CDH1} induced by DNA damage in G2 phase ensures PLK1 degradation in order to initiate efficient checkpoint response (56). In addition, CUL3–KLHL22-mediated non-degradative ubiquitination of PLK1 within the PBD regulates PLK1 localization at kinetochores and chromosome alignment (57). Defects in CUL3–KLHL22-mediated PLK1 ubiquitination result in enhanced interaction with phosphorylated proteins and consequently in sustained kinase activity towards its kinetochore substrate BUBR1, which in turn interferes with correct chromosome alignment (57). Similarly, ubiquitin-specific peptidase 16 (USP16), which is also a PLK1 substrate, deubiquitinates PLK1 to enhance its interaction with BUBR1 and to prevent premature removal of PLK1 from kinetochores (58). A delicate balance of PLK1 ubiquitination/deubiquitination is therefore required for timely PLK1 localization to kinetochores, as well as to ensure degradation of PLK1 for correct cell cycle progression.

3. Substrates and cellular processes regulated by PLK1

3.1. Cell cycle regulation

3.1.1. PLK1 in the centrosome cycle

The centrosome consists of a pair of microtubule-based centrioles enclosed in a pericentriolar matrix (PCM) that is rich in γ-tubulin complexes and functions as the major microtubule-organizing center, ensuring mitotic spindle assembly and chromosome segregation (59). Throughout the cell cycle the centrosome undergoes several

transformations, including centriole duplication, centrosome maturation and separation during mitosis, as well as centriole disengagement preceding the next duplication cycle.

The role of PLK1 in centrosome maturation, characterized by accumulation of γ-tubulin ring complexes (γ-TuRC) and other PCM proteins at the centrosome, is well established. During centrosome maturation PLK1 together with Aurora A kinase are cooperatively recruited to CEP192, a conserved scaffold protein, where PLK1 creates docking sites for γ-TuRC (Figure 2A) (60). In PLK1 depleted cells, γ-tubulin recruitment to centrosomes is strongly reduced, compromising the ability of centrosomes to establish contacts with the microtubule network (2). When Aurora A is bound to CEP192, PLK1 binds to Thr44 on CEP192 through self-priming, whereas in the absence of Aurora A, PLK1 binds to Ser955 (60) (Table 1). Notably, both Thr44- and Ser995-dependent CEP192-PLK1 interactions are required for PLK1 and γ-tubulin recruitment to centrosomes and spindle formation (60). While Aurora A kinase ensures initial activation of PLK1 and its subsequent recruitment and activation at centrosomes, PLK1 can also regulate Aurora A localization by inducing degradation of Bora (61). This makes Aurora A available to other partners, such as CENP192 or TPX2, that ensure Aurora A localization to centrosomes or microtubules, respectively (60,62). Several other PLK1-binding substrates, including CDK1-primed cenexin (a mother centriole appendage protein) or Gravin (a PCM protein), facilitate localization of PLK1 to centrosomes, promoting γ-tubulin recruitment (63,64). Centrosome separation ensures that duplicated centrosomes are separated in the G2/M transition in order to form a bipolar spindle. PLK1 is of key importance for centrosome separation, as it phosphorylates numerous key proteins involved in this process, such as kinesin EG5, and MST-2/NEK2A kinases (65). PLK1 is absolutely indispensable for the initiation of centrosome maturation and for the formation

of a bipolar spindle, as PLK1 inhibition leads to monopolar spindle formation with impaired microtubule organizing activity (66–68).

At the end of mitosis, PLK1 activity contributes to centriole disengagement involving the separation of daughter and mother centriole, which is an important prerequisite for the subsequent round of centriole duplication. It is a strictly time-controlled event, as premature centriole disengagement in G2 or early mitosis will lead to spindle multipolarity and improper chromosome segregation. PLK1-mediated phosphorylation of several substrates allows separase to cleave and remove cohesive proteins (such as cohesin, pericentrin) holding two centrioles together (69–72). In agreement, inhibition of PLK1 activity blocks centriole disengagement during mitotic exit (69,70).

In quiescent cells, mature centrioles can act as a basal body and anchor cilia, a special sensory antenna, to the membrane. Cilia disassembly is required to allow the duplication of centrioles and subsequent centrosome separation before mitotic entry. PLK1 activity is necessary to promote disassembly of the primary cilia by phosphorylating DZIP1 (a protein required for ciliogenesis), enabling the dissociation of a multiprotein complex that maintains a functional cilium (73). Additionally, PLK1 activates histone deacetylase 6 (HDAC6), leading to cilia deacetylation and resorption (74). While Wang and colleagues suggested that PLK1 can directly interact with and activate HDAC6, another study showed that PLK1 regulates the Aurora A-mediated HDAC6 activation by controlling the stability of human enhancer of filamentation 1 (HEF1), a scaffold protein that activates Aurora A to induce cilia disassembly (75)(76).

Taken together, PLK1 is shown to play a vital role in the centrosome life cycle during cell division, by phosphorylating key centrosomic proteins and targeting proteins to the

centrosomes. Beyond mitosis, PLK1 also participates in a strictly controlled process of ciliogenesis, where aberrant ciliary functions lead to several ciliopathies including renal cysts, diabetes hypertension, neuronal disorders and the Bardet-Biedl syndrome (77).

3.1.2. PLK1 in mitotic entry

During the G1 and S phase of the cell cycle, mitotic kinases are kept inactive to prevent premature entry into mitosis. The signaling cascade leading to activation of mitotic kinases is triggered by Cyclin B/CDK1-dependent phosphorylation of Bora that ultimately allows Aurora A to activate PLK1 (78,79). The CDK1 kinase itself is kept inactive by the kinases WEE-1 and MYT-1 (Figure 2B) (37,41). Activated PLK1 binds to CDK-primed WEE-1 and together with CK2 phosphorylates WEE-1, targeting WEE-1 to proteasome-dependent degradation (37). PLK1 also phosphorylates MYT-1 during G2/M transition and inhibits its kinase activity, further contributing to the feedforward loop aimed at the activation of mitotic kinases and mitosis entry (41,80). In parallel, PLK1 phosphorylates CDC25C, a phosphatase responsible for activation of the Cyclin B1/CDK complex, which promotes nuclear localization of CDC25C at prophase during the G2/M transition (78,79,81). Additionally, Cyclin B1 gets phosphorylated by PLK1 allowing nuclear translocation of cyclin B1 (40,81). Consequently, the cyclin B1/ CDK1 complex is activated and the cell proceeds to mitosis. Another interesting study showed that CYLD, a tumor suppressor and deubiquitinating enzyme, interacts with PLK1 and is required for timely entry into mitosis (82). CYLD localizes to interphase microtubules but translocates to the midbody during telophase. Both PLK1-depleted and CYLD-depleted cells show similar phenotypes during mitotic entry, characterized by delayed CDC25C phosphorylation and stabilization of the APC/C inhibitor

EMI-1, while overexpression of either CYLD or PLK1 leads to accumulation of multinucleated cells (82). CYLD-mediated PLK1 regulation is independent of the known role of CYLD as a negative regulator of nuclear factor-κB (NF-κB) signaling. CYLD was proposed to regulate PLK1 polyubiquitination by for instance removing K63-polyubiquitin chains on PLK1 or its upstream regulators (82).

3.1.3. PLK1 in establishment of kinetochore-microtubule attachments, spindle orientation and chromosome stability

Equal mitotic chromosome segregation is of key importance for genome integrity and is controlled by the spindle assembly checkpoint (SAC). The SAC inhibits mitotic progression until all sister chromatids become attached via their kinetochore to microtubules emerging from opposite spindle poles. The first scientific evidence that PLK1 is of key importance for spindle assembly formation came in 2004 from Van Vugt et al., who showed that PLK1-depletion resulted in mitotic arrest and spindle defects, as chromosomes failed to align (2). In metaphase, PLK1 gets recruited to the kinetochores by several proteins including BUBR1, BUB1, PBIP1, NUDC, CENP-E and INCENP (Figure 2C) (35,38,83–86). For example, PLK1 phosphorylates PBIP1, which self-regulates the PLK1-PBIP1 interaction to timely localize PLK1 to the kinetochores, while later in mitosis PLK1 mediates PBIP1 degradation (35). PLK1 also binds to CDK1-primed INCENP, an inner centromere protein which also gets phosphorylated by Aurora B, a kinase known for correcting kinetochore-microtubule attachments (85). In this way an INCEP/PLK1/Aurora B complex is formed which ensures metaphase to anaphase transition (84).

The key step in SAC signaling is the binding of the Monopolar Spindle 1 (MPS1) kinase to unattached kinetochores, where it promotes the formation of the mitotic checkpoint complex (MCC) that prevents the pre-mature activation of APC/C and mitosis exit (87). MPS1 then phosphorylates MELT (Met-Glu-Leu-Thr) repeats in the scaffold protein KNL-1, mediating the recruitment of the MAD1:C-MAD2 and BUB1/BUB3-BUB3/BUBR1 complexes to kinetochores, which cooperate to form the MCC (88). PLK1 interaction with BUB1 has been shown to play a major role in supporting MCC function (86). Also, hyperphosphorylation of BUBR1 by PLK1 is important for the establishment of stable kinetochore-microtubule interactions and for proper chromosome alignment at the spindle equator (38). PLK1 phosphorylates two MPS1 substrates, KNL-1 and MPS1 itself, thereby enhancing the catalytic activity of MPS1 and MCC formation (86,89). When kinetochores have attached properly, KNL-1 phosphorylation has to be reversed to allow disassembly of MCC and cell cycle progression to anaphase. PLK1 removal from the BUB complex is promoted by the kinetochore phosphatases PP1 and PP2-B56 and is required to prevent PLK1 from continuously phosphorylating KNL-1 and SAC silencing (90). Disintegration of MCC finally allows activation of APC/C. The APC/C is a multi-subunit ubiquitin ligase that targets several mitotic proteins for proteasomal degradation, allowing cells to enter anaphase where the separation of the sister chromatids occurs. PLK1 further contributes to APC/C activation by promoting phosphorylation-dependent degradation of EMI1, a major APC/C inhibitor in mitotic cells (Figure 2D) (87,88,91). In agreement, inactivation of PLK1 results in EMI-1 stabilization during mitosis (91). As PLK1 is overexpressed in several types of cancer cells (discussed below), very high levels of PLK1 might be sufficient to activate the APC/C before ensuring that chromosomes are correctly aligned to the mitotic spindle and

thus leading cells to premature progression to anaphase, resulting in genome instability and contributing to tumorigenesis.

While PLK1 localized at the kinetochores helps surveying kinetochore-microtubule attachments, PLK1 found at centrosomes coordinates correct spindle orientation. During metaphase, PLK1 phosphorylates NuMA, an adaptor protein for anchoring dynein required for proper mitotic spindle orientation, and negatively regulates cortical localization of NuMA (92). This seems to be essential for correct spindle orientation, since PLK1 inhibition in metaphase cells leads to spindle orientation defects (92). Another recent finding shows that PLK1 interacts with the centriolar protein CEP76, which prevents the activation of PLK1 in the cytoplasm during mitosis (93). Depletion of CEP76 induced ectopic aggregation of hyper-phosphorylated PLK1 in the cytoplasm, accompanied by defective spindle orientation and mitotic delay. Notably, this phenotype was rescued by PLK1 inhibition (93).

PLK1 is also involved in maintaining chromosome stability via at least two mechanisms. One example is its interaction with BRCA2, a tumor suppressor and DNA repair protein, which ensures alignment of chromosomes to the metaphasic plate (8). In particular, PLK1 binds and phosphorylates BRCA2, enabling the formation of the BRCA2-pBubR1-PLK1-PP2A complex at the kinetochores, which allows for stable kinetochore-microtubule interactions and chromosome stability. Incapability to phosphorylate BRCA2 leads to chromosome misalignment defects that prolong mitosis and subsequently increase errors in chromosome segregation and can lead to aneuploidy (8). Another study reported that during mitosis PLK1 is recruited to a RIPK1/FADD/Caspase 8/cFLIP multiprotein complex (ripoptosome) via interaction with the kinase RIPK1, where PLK1 gets cleaved by Caspase 8 (7). Ripoptosome formation negatively regulates PLK1-mediated phosphorylation of its downstream

substrates, for instance BUBR1, in this way fine-tuning PLK1 localization and activity. Importantly, Caspase 8 and RIPK1-mediated regulation of PLK1 is crucial in order to maintain chromosome stability, as depletion or inhibition of RIPK1 or Caspase 8 results in chromosome alignment defects (7). Taken together, PLK1 has a vital role in the establishment and maintenance of kinetochore-microtubule attachments, as well as correct spindle orientation. Deregulation of PLK1 activity leads to increased chromosome segregation errors and aneuploidy, which ultimately promotes carcinogenesis, a topic which will be discussed in the next sections.

3.1.4. PLK1 in cytokinesis and abscission

Cytokinesis concludes mitosis producing two sister cells. In animal cells, cytokinesis starts in anaphase with the assembly of an actomyosin-based contractile ring, which marks the plane of cell division, leading to the formation of the cleavage furrow. The furrow rapidly deepens and spreads around the cell until it completely divides the cell in two. This event is preceded by the formation of the spindle midzone consisting of the interpolar microtubules bundled between the separating sister chromatids. As the furrow spreads, the microtubule bundles form a cytoplasmic bridge, with the proteinaceous midbody in its centre promoting final abscission (94). Defective cytokinesis may result in cells with abnormal ploidy and genomic instability, which promotes tumor development. Consequently, similarly to the entire cell cycle process, cytokinesis is spatially and temporally regulated. The centralspindlin protein complex, which consists of two major components, HsCYK-4 and Mitotic kinesin-like protein 1 (MKLP1), is a key motor complex nucleating microtubule bundling at midzone that recruits

the Rho guanine nucleotide exchange factor ECT2, promoting the activation of RhoA GTPase responsible for the contractile ring assembly (95,96).

Before the discovery of fast acting chemical PLK1 inhibitors (discussed below) the role of PLK1 in cytokinesis was difficult to establish, because depletion of PLK1 causes cells to arrest at the early stages of mitosis and complicates research on the late stages of mitosis and cytokinesis. For instance, using PLK1-depleted cells, Van Vugt and colleagues concluded that PLK1 has no role in the initiation of cytokinesis and midbody formation, while nuclear division is highly asymmetric due to monopolar spindle formation (2). However, a later report demonstrated that PLK1 inhibition in anaphase prevents cytokinesis, RhoA accumulation in the midzone and contractile ring formation (97). More specifically, PLK1 inhibition at anaphase selectively abolished the recruitment of ECT2 to the central spindle and abrogated ECT2-HsCYK-4 interaction (97). PLK1 binds and phosphorylates HsCYK-4 at the midzone, creating a docking site for ECT2 (Figure 2E) (36,98). Similar to the localization of PLK1 to centrosomes and kinetochores, PLK1 localization to midzone is facilitated by interactions of the PLK1 PBD domain with several midzone proteins. For example, PLK1 was shown to interact with MKLP2 kinesin and phosphorylate it, which is required for the spatial restriction of PLK1 during anaphase and telophase (99). Furthermore, PLK1 binds to microtubule-associated protein regulating cytokinesis (PRC1) in anaphase, which is prevented in metaphase by CDK1-mediated phosphorylation of PRC1 at a site adjacent to the PLK1 docking site (100). Yet, in PRC1-depleted cells where spindle midzone is disrupted, cleavage furrow ingression can occur independently of PLK1 in an alternative pathway dependent on Aurora B and centralspindlin at the equatorial cortex (101). Furthermore, it has been suggested that PLK1-dependent phosphorylation of PRC1 enables the release of centralspindlin from the spindle midzone to the equatorial cortex (101). PLK1 also

phosphorylates abscission-promoting factor, CEP55, which negatively regulates the central spindle recruitment of CEP55 (102). Therefore, PLK1 degradation is required for the timely recruitment of CEP55 to the midbody, providing a mechanism to control the timing of abscission(103). When the activity of PLK1 is inhibited, CEP55 is prematurely recruited to the midbody but fails to complete cytokinesis (103).

Taken together, PLK1 has diverse roles through the entire cell cycle, from interphase to cytokinesis: it controls centrosome maturation and separation, is involved in the establishment and maintenance of kinetochore-microtubule attachments, regulates the metaphase to anaphase transition and contributes to proper spindle orientation, as well as cytokinesis and abscission. Deregulated PLK1 activity results in numerous mitotic defects and contributes to chromosome instability which can be exploited by tumor cells.

3.2 Role of PLK1 in DNA replication, DNA damage response and genome stability

3.2.1. PLK1 in DNA replication

PLK1 has been extensively studied for its role in mitosis, however several reports also point to the role of PLK1 in DNA replication. DNA replication starts with the orderly assembly of the pre-replicative complex (pre-RC) at the origins of replication initiated by the binding of multiprotein origin recognition complex (ORC). ORC further recruits CDC6 and CDT1 components, which enable the subsequent engagement of the minichromosome maintenance complex 2-7 (MCM2-7) and allow DNA replication in the S phase (104). PLK1 has been found to interact with and phosphorylate several components of the pre-RC complex including ORC2 (105), the proteins of the MCM2-7 (106) and the human histone acetyltransferase binding to ORC1 protein (HBO1) (Figure 3A) (107). For example, CDK1 primes HBO1 at Thr85/88, allowing PLK1 to dock on and phosphorylate HBO1, which is

crucial for pre-RC assembly, as overexpression of the non-phosphorylatable HBO1 Ser57Ala mutant leads to impaired pre-RC formation and DNA replication (107). Furthermore, PLK1 phosphorylates ORC2, another component of the pre-RC complex, upon cell treatment with DNA replication inhibitors such as low doses of UV, hydroxyurea and aphidicolin, which contributes to the pre-RC maintenance under DNA replication stress but not under normal conditions (105). Similarly, PLX1, the Xenopus orthologue of human PLK1, is recruited to the MCM complex via the phosphorylated MCM2 protein and is required for DNA replication under stress conditions (108). Consequently, it has been suggested that PLK1 activity contributes to S phase progression in cancer cells, due to the fact that cancer cells, having high genome instability, regularly encounter DNA replication stress (109). Indeed, in cancer cells, PLK1 depletion during G1/S phase slowed DNA synthesis, disrupted the binding of MCM protein to chromatin and resulted in high levels of pre-RC inhibitor geminin (110). Under normal conditions PLK1 mediates phosphorylation-dependent degradation of EMI-1 and consequent activation of the APC/C, which in turn promotes degradation of geminin licensing DNA replication in the S phase. However, EMI1 levels were increased in PLK-1 depleted cells causing APC/C inhibition, stabilization of geminin and disruption of MCM binding (110). On the other hand, in untransformed human cells PLK1 activity is actually restricted in S phase by DNA replication (111). It has been proposed that DNA replication promotes activation of checkpoint kinases, which in turn restrict mitotic kinases to prevent premature entry into mitosis and CDK1-mediated DNA damage (111). Also, PLK1 methylation on Lys209 by methyltransferase G9a was shown to increase in S phase and inhibit PLK1 activation by preventing Thr210 phosphorylation. It is possible that DNA replication-relevant PLK1 interactions with its substrates are taking place in mitosis and contribute to DNA replication licensing, while PLK1 activity during S phase itself is kept to a

minimum. Indeed, PLK1-HBO1 interaction takes place only in nocodazole-arrested cells, but not in other stages of the cell cycle (107). Interestingly, PLK1 interaction with a centrosomal protein FOR20 that recruits PLK1 to the centrosomes was suggested to license cell cycle progression to S phase (112). Depletion of FOR20 induced S phase defects, which were rescued by forced centrosomal localization of PLK1, independent of its catalytic activity (112). Collectively, these data suggest that PLK1 has a scaffold function at the centrosomes necessary for S phase progression. Alternatively, PLK1 sequestration at the centrosomes during S phase might ensure that its activity is kept to a minimum in the nucleus allowing DNA replication.

Finally, PLK1 was also shown to phosphorylate DNA topoisomerase II α, an enzyme that helps relaxing DNA molecules overwound in the process of replication and promotes chromosome disentanglement (113). Overexpression of non-phosphorylatable DNA topoisomerase II α phospho-mutants led to a G1/S phase arrest, probably due to activation of the DNA damage checkpoint and reduced topoisomerase activity (113).

3.2.2. PLK1 in DNA Damage Response and Genome Stability

DNA damage can occur throughout the entire cell cycle process and if left unrepaired or repaired improperly, it can lead to mutations and eventually carcinogenesis. Specific safety mechanisms, collectively named the DNA Damage Response (DDR), detect DNA lesions, signal DNA damage to pause cell cycle and promote DNA repair (114). Surveillance checkpoints ensure the proper progression of the cell cycle. The G1/S checkpoint ensures that DNA is not damaged before cell cycle progression to S phase and DNA replication. Here, the kinases ATM and ATR act as crucial sensors of DNA damage and once activated,

phosphorylate downstream checkpoint kinases CHK1 and CHK2 (115). The checkpoint kinases, in turn, phosphorylate phosphatase CDC25A, marking it for ubiquitination and degradation, so that the cyclin E/CDK2 complex remains inactive and the cells are retained in G1 phase (Figure 3B) (116). Similarly, cells are examined at the G2/M checkpoint for DNA damage or incomplete replication prior to entering mitosis. Again, ATM/ATR-mediated signaling ensures CDC25 phosphorylation preventing Cyclin B-CDK1 complex formation, which is responsible for mitotic entry (117). The last checkpoint, as discussed in the previous chapters, is the SAC, which is activated in case of improper chromosome alignment and inhibits the APC/C complex responsible for progression through anaphase.

When DNA damage occurs in interphase, ATM/ATR-dependent checkpoint pathways inhibit PLK1 phosphorylation and activation in order to prevent cell entry into mitosis while carrying damaged genetic material (Figure 3B) (118,119). More specifically, DNA damage interferes with the interaction of Aurora A and the Bora/PLK1 complex, preventing Aurora A-mediated PLK1 activation (120). DNA damage also leads to phosphorylation of Bora at Thr501 by ATR, which results in the proteasome-mediated degradation of Bora, significantly lowering the amounts of Bora (121). Additionally, cell exposure to genotoxic agents that cause DNA damage leads to the accumulation of inactive Lys209 monomethylated PLK1 that further contributes to the restraining of PLK1 activity during DNA damage repair (122). This PLK1 methylation was shown to be necessary for timely removal of DNA binding proteins, RPA2 and RAD51, although the exact mechanism is still unclear (122). In fact, PLK1 was suggested to actively contribute to DDR by phosphorylating several proteins essential for DNA double-strand break repair via homologous recombination, such as recombinase RAD51 and BRCA1, which facilitates their recruitment to the sites of DNA damage (123–125). PLK1 inhibition shortly before DNA damage sensitizes cells to ionizing radiation and

decreases double-strand break repair by homologous recombination and results in reduction of BRCA1 foci formation (125). Another study showed that BRCA1, in turn, downregulates PLK1 activity via regulating the phosphorylation and abundance of Bora (126). However, it is unclear how and when PLK1 gets activated during homologous recombination in response to DNA damage. It might only come to play during prolonged replication stress where PLK1 contributes to cell transformation (see below).

DNA damage occurring in G2 phase has also been shown to reactivate APC/C^{CDH1}, which is normally active in late mitosis and S phase (56). APC/C^{CDH1}, in turn, induces degradation of PLK1 ensuring successful DDR during G2 (56). On the other hand, PLK1 activation is essential for checkpoint recovery (127) in order to induce proteasome-mediated degradation of Claspin that controls CHK1 activation by ATR (56,128).

DNA damage can also occur during mitosis, in which case mitotic kinases are deactivated and, as a result, cells can skip late stages of mitosis and cytokinesis ending up with 4N DNA content (129). A recent study shows that deactivation of PLK1 during mitotic checkpoint is due to the activation of ATM/CHK kinases that in turn inhibit the Greatwall kinase (130). The latter regulates DNA damage responses in the G2 phase and controls the timing of mitotic entry after DNA damage by activating the inhibitors of the PP2A-B55 phosphatase complex (131,132). Loss of phosphorylation of Greatwall during early mitotic DNA damage leads to the activation of the PP2A-B55 complex, which in turn dephosphorylates PLK1 (130). Another study, however, found that PLK1 is not necessary for the CDK1-mediated Greatwall export to the cytoplasm before nuclear envelope breakdown (133). Furthermore, there was no difference in PLK1 activity in any phase of cell cycle when Greatwall knockout cells were

compared to wild type cells (134), questioning the role of PLK1 in the DNA damage response during mitosis.

Untimely activation of PLK1 during DDR might override the DNA damage checkpoint, allowing cells to continue with the cell cycle, which might lead to genome instability and cell transformation. Thus, PLK1 might contribute to genome instability in multiple ways. PLK1 has been shown to promote error-prone microhomology-mediated end joining via phosphorylation of CtIP, an interaction partner of the MRE11-RAD50-NBS1 (MRN) complex, which together recognize double-strand DNA breaks and initiate repair pathways (135,136). In particular, in nocodazole-arrested cells further treated with a DNA damage-inducing agent, PLK1 phosphorylated CDK1/Aurora A-primed CtIP at Ser723 and increased the activity of microhomology-mediated end joining (136). Furthermore, PLK1 has been shown to phosphorylate MRE11 at Ser649 enabling subsequent phosphorylation by CK2, which together inhibit the loading of the MRN complex to damaged DNA and lead to the premature termination of the DNA damage checkpoint (11). Similarly, PLK1-mediated phosphorylation of the 53BP1 protein at Ser1618 inhibits the binding of 53BP1 to ubiquitinated Histone 2A, preventing correct localization of the former to the sites of DNA breaks and DNA damage repair (137). Recently PLK1 has been shown to negatively regulate expression of NOTCH1 at G2/M transition (138). NOTCH1 signaling is involved in a broad range of biological processes and, depending on the context, has a tumor suppressive or an oncogenic role (139). During the G2 damage checkpoint, while PLK1 is inhibited, NOTCH1 expression is upregulated. Finally, PLK1 is known to interact with p53 - a master tumor suppressor protein activated in response to DNA damage by checkpoint kinases in order to promote cell cycle arrest and, if necessary, apoptosis (140). p53 also acts as a counterbalance to PLK1 activity by negatively regulating PLK1 expression via several

mechanisms (141–143). Complex interplay between PLK1 and p53 ensures timely PLK1 activation when DNA damage is repaired, allowing cell cycle progression, whereas when DNA is severely damaged, p53 transcriptionally inhibits PLK1 and promotes apoptosis of defective cells. Disruption of this mutual regulation leads to the oncogenic transformation of cells. The role of the PLK1-p53 axis in cancer will be discussed in more detail in one of the following sections.

3.3 PLK1 beyond cell cycle

3.3.1. PLK1 in autophagy and apoptosis

Autophagy is a highly conserved and adaptive self-degradative process that ensures optimal redistribution of energy sources at times of cellular stress, including nutrient deprivation, growth factor depletion, infection and hypoxia**.** Deregulation of autophagy leads to many pathologies including infections, cancer and neurodegenerative disorders. Furthermore, because autophagy is an adaptive mechanism geared towards maintaining cell homeostasis, it acts as a double-edged sword in cancer. Autophagy has tumor-suppressing properties, as it degrades potentially harmful agents or damaged organelles, thus preventing proliferation of cells with damaged DNA (144). On the other hand, autophagy has been shown to promote cancer cell proliferation and tumor growth (145). Ser/Thr kinase mTOR (also known as mammalian target of rapamycin) is a key kinase that together with the regulatory-associated protein of mTOR (RAPTOR) forms mTOR complex 1 (mTORC1) and inhibits autophagy under normal conditions (146). mTORC1 activation requires its translocation to lysosomes, where it prevents autophagy initiation (147). PLK1 was shown to co-localize and interact with mTORC1 at lysosomes, as well as to directly phosphorylate RAPTOR *in vitro* (9).

PLK1 inhibition promoted mTORC1 targeting to the lysosomes and reduced autophagy in non-mitotic cells (9). In human glioma cells, PLK1 expression is elevated and knock down of PLK1 enhanced mTORC1 activity and induced caspase-dependent apoptosis (148). Similarly, PLK1 inhibition sensitized breast cancer cells to radiation by inhibiting autophagy (149). Additionally, it has been reported that PLK1 facilitates autophagy in osteosarcoma cell lines by stabilizing MYC expression, an oncogenic transcription factor often upregulated in tumors that among other functions promotes autophagy (10). Remarkably and in contrast to the above-mentioned findings supporting a facilitating effect of PLK1 in autophagy, PLK1 inhibition (both by using small molecule PLK1 inhibitors, but also by silencing PLK1 using siRNA) in acute myeloid leukemia (AML) cells resulted in induction of autophagy via dephosphorylation of mTORC1 (150). The reason for these conflicting results remains unclear but may indicate that the role of PLK1 in autophagy can be dependent on the cell type or specific experimental conditions.

As the main goal of PLK1 is to promote cell cycle progression and consequently cell proliferation, PLK1 often indirectly contributes to protecting cells from cell death pathways. For example, by inhibiting p53 or inducing its degradation, PLK1 blocks p53-induced apoptosis (151,152). However, some reports also suggest that PLK1 might have more direct anti-apoptotic activities (153). Caspase 8 plays a central role in directing apoptosis in response to extrinsic stimulation of death receptors such as Fas. PLK1 was shown to interact with and phosphorylate CDK1-primed pro-caspase 8 in mitotic cells, interfering with Caspase-8 auto-activation and decreasing cell sensitivity towards the extrinsic cell death pathways during mitosis (153). Conversely, PLK1 inhibition with the small molecule inhibitor BI2536 significantly lowered the threshold of several cancer cell types to Fas-induced cell death (153). In contrast to this reported inhibitory effect of PLK1-mediated Caspase-8

phosphorylation on Fas-induced cell death, PLK1-mediated phosphorylation of Fas-associated death domain (FADD) in response to paclitaxel treatment has been shown to promote cell death (154), and in turn mediates PLK1 proteasomal degradation creating a negative feedback loop. This opposite outcome of PLK1-mediated phosphorylation of Caspase-8 and FADD is rather remarkable as both are known to be part of the same death-inducing signaling complex. A regulatory role of PLK1 in cell death was also described in other conditions. For example, detachment of esophageal squamous cell carcinoma cells induces NF-κB-dependent PLK1 upregulation, leading to the inhibition of β-catenin degradation and protection from detachment-induced cell death (155). Similarly, NF-κB- and AKT-dependent PLK1 upregulation and β-catenin stabilization was also reported in pancreatic ductal epithelial cells upon insulin stimulation and was suggested to contribute to pancreatic cancer (156). Taken together, in certain conditions, PLK1 ensures cell survival by inhibiting cell death pathways and encouraging autophagy in times of stress.

3.3.2. PLK1 in epithelial mesenchymal transition (EMT)

Epithelial mesenchymal transition (EMT) is a mechanism in which cells lose their epithelial cell characteristics such as cell-cell junctions, cell polarity and cobblestone morphology and acquire mesenchymal characteristics that facilitate invasiveness and motility. Cells that undergo EMT become multipotent stromal cells, capable of differentiating into a variety of cell types (157). Although EMT is indispensable for several developmental processes and wound healing, it can also help cancer cells to avoid cell death and promote metastasis. On the molecular level, loss of E-cadherin accompanied by increase of mesenchymal markers including N-cadherin and vimentin is indicative of EMT. Available data point to a possible

role for PLK1 in driving EMT. PLK1 overexpression (but not a kinase dead PLK1 mutant) in prostate epithelial cells promotes oncogenic transformation and EMT, while PLK1 downregulation in metastatic prostate cancer cells enhanced epithelial characteristics, reversed the EMT and inhibited cell motility (158). These effects were triggered by PLK1-mediated phosphorylation of cRAF, which in turn induces the MEK/ERK cascade ultimately activating ZEB1 and ZEB2 transcription factors enabling the expression of EMT genes (158). Furthermore, PLK1 was shown to promote EMT also in other cancer cells including gastric carcinoma cells (159) and non-small cell lung cancer cells (NSCLC) (160,161). In particular, in gastric carcinoma cell lines, PLK1 facilitated invasion via phosphorylation of AKT, which is known to regulate the stability of another EMT transcription factor Snail, while PLK1 silencing reversed EMT (159,162). In a large-scale integrated analysis of gene and protein expression in several NSCLC cell lines, cell lines with high EMT gene signature scores exhibited higher sensitivity to PLK1 inhibition than epithelial cells (160). Active PLK1, phosphorylated at Thr210, was also upregulated in TGF-β-treated NSCLC and PLK1 knockdown or PLK1 inhibitor treatment blocked TGF-β-induced tumorigenic and pro-metastatic activity in NSCLC (161). Collectively, these data illustrate yet another facet of PLK1 function that enables tumor progression.

3.3.3. PLK1 in inflammatory signaling

PLK1 overexpression or activation in nocodazole-arrested cells was shown to inhibit the transcriptional activation of NF-κB in response to various inflammatory stimuli (IL-1β, TNF) or overexpression of specific NF-κB signaling proteins (RIPK1, TRAF2 and MyD88) (155,156,163,164). Mechanistically, PLK1 was shown to phosphorylate IκB kinase (IKK)β at

Ser740, preventing the formation of a functional IKK complex that mediates the phosphorylation and subsequent proteasomal degradation of the NF-κB inhibitor IκBα, leading to NF-κB activation (Figure 4A). Alternatively, it has been suggested that PLK1 can also inhibit IKK activity and subsequent NF-κB activation by inhibiting the ubiquitination of the IKK adaptor protein NEMO, to which it is recruited via PLK1 binding to TRAF-associated NF-κB activator (TANK) (164). PLK1 also regulates NF-κB and IRF3 transcription factor activation in response to intracellular sensing of viral RNA and leading to the expression of proinflammatory proteins and antiviral type I interferons, respectively (Figure 4B). More specifically, in this RNA sensing signaling pathway, the PBD domain of PLK1 interacts with the mitochondria-bound adaptor protein MAVS in two different ways: the PBD docks at the N-terminal end of MAVS in a phospho-dependent manner, but also docks to the C-terminal domain of MAVS independently of MAVS phosphorylation (165). The latter interaction disrupts the subsequent recruitment of TRAF3 to MAVS, which is essential for activation of an alternative IKK complex responsible for IRF3 phosphorylation (165). The real implications of PLK1 in regulating inflammatory signaling are still poorly understood and await further exploration. It is currently also unclear whether the modulation of inflammatory signaling is linked to its cell cycle regulatory role or if it reflects another role for PLK1 beyond the cell cycle.

4. PLK1 in disease

4.1 PLK1 in cancer

4.1.1. PLK1 as a tumor promotor

Considering the prominent role that PLK1 plays in cell cycle and cell proliferation, as well as in suppressing apoptosis and promoting EMT, it is not unexpected that PLK1 can act as a strong tumor promotor. The tumor promoting function of PLK1 was first reported in 1997, when it was shown that overexpression of PLK1 in the murine fibroblast cell line NIH 3T3 leads to reduced serum growth requirements, loss of contact inhibition, and anchorage-independent growth *in vitro*. Furthermore, PLK1 transfected cells formed tumors after subcutaneous injection in nude mice (166). PLK1 is overexpressed in a variety of cancers, including breast invasive carcinoma (167), hepatocarcinoma (168), colon adenocarcinoma (169), lung squamous cell carcinoma (160), prostate adenocarcinoma (170), gastric cancer (171), ovarian cancer (172), melanoma (173). Furthermore, PLK1 levels often correlate with poor prognosis, tumorigenicity and aggressiveness, and PLK1 expression is therefore used as a prognostic marker (4).

As briefly discussed above, PLK1 can regulate the transcriptional ability and stability of the major tumor suppressor p53 at multiple levels (152). The *TP53* gene is mutated in about 50% of all tumor malignancies. In addition, many tumors that do not contain mutations in the *TP53* gene, have inactivated p53 in several ways, resulting in impaired p53 function in tumors (174). As there is a reciprocal regulation between PLK1 and p53, cells that have lost p53 and have increased PLK1 expression levels are therefore at high risk of becoming oncogenic. Additionally, PLK1 induces p53 degradation in several ways. For example, PLK1 phosphorylates GTSE1, a negative regulator of p53-required for G2 checkpoint recovery, and promotes its nuclear localization (Table 2). As a result, p53 is shuttled back to the cytoplasm where it gets degraded (151). In addition, PLK1 is capable of phosphorylating the p53 inhibitor protein MDM2, thereby promoting the MDM2-mediated degradation of p53 (143). PLK1 also phosphorylates Topors and inhibits Topors-mediated sumoylation of p53,

enhancing p53 ubiquitination and targeting it for degradation (175). Finally, PLK1 phosphorylates the p53-interacting and -stabilizing protein NUMB, promoting its degradation and leading to p53 destabilization (12,176). Consequently, tumors carrying the NUMB phospho-mutant are more sensitive to chemotherapy (12).

PLK1 can also inactivate another tumor suppressor and a direct inhibitor of the PI3K/AKT pathway, phosphatase and tensin homolog (PTEN) (177). PTEN phosphorylation reduces its activity and nuclear localization, leading to PI3K/AKT activation, which drives cell proliferation and increased aerobic glycolysis (178). PI3K/AKT signaling, in turn, can contribute to PLK1 activation. More specifically, during mitosis PLK1 binds to the 14-3-3γ protein, which promotes PLK1 catalytic activation independently of Thr210 phosphorylation, but requires previous PLK1 phosphorylation on Ser99 by PI3K and AKT (49). In pancreatic cancer cells, PI3K/AKT inhibition leads to cell apoptosis and represses pancreatic tumor growth by downregulating PLK1 (179). Combined inhibition of PI3K/AKT and PLK1 in pancreatic carcinoma makes cells more sensitive towards chemotherapeutic agents (180). Conversely, loss of PTEN leads to PLK1 overexpression in murine prostate tissues that enables cell adaptation to mitotic stress and survival, ultimately inducing prostate cancer in mice, while reintroduction of wild type PTEN in PTEN-depleted prostate cancer cells reduced their survival dependence on PLK1 (177). PLK1 is often overexpressed in prostate cancer, where it is also known to activate androgen receptor, a crucial effector of prostate cancer development and progression, and is linked to higher tumor grades (177,181). Dual inhibition of PLK1 and androgen signaling blocked prostate tumor growth and reduced PSA levels in serum (181). Other examples of PLK1 tumor suppressor targets include PLK1 phosphorylation-dependent degradation of REST in triple negative breast cancer (167) or SUZ12 and ZNF198 in hepatitis B virus (HBV)-mediated hepatocarcinoma (182,183). PLK1

also inhibits transcriptional activity of FOXO1, a key tumor suppressor which promotes apoptosis in prostate cancer cells. Conversely, when PLK1-mediated phosphorylation of FOXO1 is inhibited, the pro-apoptotic function of FOXO1 is restored, leading to tumor repression in advanced prostate cancer cells (184).

In addition to inhibiting tumor suppressors, PLK1 also regulates stability of several oncogenes. For example, PLK1 regulates the expression of MYC family oncoproteins by enhancing their stability (10,185). In particular, PLK1 phosphorylates the E3 ubiquitin ligase FBW7, which is responsible for the proteasomal-mediated degradation of MYC. As a result, FBW7 itself is targeted for ubiquitination and degradation, thus increasing the levels of N-MYC, but also Cyclin E and the anti-apoptotic protein MCL1 (185). On the other hand, N-MYC directly activates PLK1 transcription, creating a positive feed-forward loop (185,186). PLK1 inhibition significantly impaired survival of cells overexpressing MYC as well as subcutaneous xenografts derived from tumor cells from neuroblastomas, small cell lung carcinomas and aggressive B cell lymphomas *in vivo* (185,186). This effect was even more pronounced upon simultaneous use of an inhibitor of BCL2, another anti-apoptotic protein deregulated in many tumors (185,186). A second mechanism by which PLK1 was found to sustain MYC stability, is via activating the AKT/GSK3β kinase circuit, known to stabilize MYC in various tumors (186,187).

A recent study also shows that in nasopharyngeal carcinoma, PLK1 phosphorylates oncoprotein KLF4, which in turn results in the recruitment of the E3 ubiquitin ligase TRAF6, leading to K63-ubiquitination and stabilization of KLF4. In turn, KLF4 enhances the expression of TRAF6, thereby initiating a feed-forward loop (188). Consequently, PLK1 inhibition reduced tumor growth in a mouse model bearing nasopharyngeal carcinoma

xenografts (188). Additionally, PLK1 and STAT3 were also found to positively regulate each other's expression and were significantly elevated in glioblastoma and esophageal cancer cells correlating with poor prognosis (189,190). Dual STAT3 and PLK1 inhibition repressed glioblastoma cell invasion and promoted cell apoptosis, through MYC inhibition (189).

Finally, besides regulating the activity of tumor suppressors and oncogenes, PLK1 has a novel function in regulating metabolism in cancer cells. In addition to the PLK1/PI3K/AKT-dependent aerobic glycolysis mentioned above (179), PLK1 also activates the pentose phosphate pathway, which is aberrantly activated in many cancers (191). In particular, PLK1 interacts with and directly phosphorylates glucose-6-phosphate dehydrogenase (G6PD), leading to its dimerization-induced activation and promoting cancer cell growth. Moreover, PLK1 inhibition abrogated the G6PD-mediated tumor growth both in cell lines and in mice (191).

4.1.2. PLK1 as a tumor suppressor

Interestingly, despite its prominent oncogenic activities, PLK1 can also act as a tumor suppressor in certain circumstances. Deletion of PLK1 in mice resulted in embryonic lethality, while mice heterozygous for the *PLK1* allele had 3 times higher frequency of developing tumors compared to wild type mice, highlighting once more that normal PLK1 levels are crucial for correct cell division (192). A recent study by De Carcér et al. showed that inducible PLK1 overexpression in mice reduces KRAS- or HER2-induced mammary gland

tumor development (193). Furthermore, PLK1 overexpression was shown to correlate with higher survival of patients with specific breast cancer types (193). Additionally, PLK1 was shown to regulate estrogen-dependent expression of genes with tumor suppressive properties that positively correlate with positive outcomes in breast cancer patients (194). Similarly, high PLK1 levels correlated with improved survival of colon cancer cells carrying specific *APC* mutations. More specifically, PLK1 inhibition in colon cells expressing mutant APC-ΔC compromises SAC function due to reduced localization of BUBR1 and MAD1 to the kinetochores, increasing chromosomal abnormalities and leading to a higher number of intestinal tumors in $Apc^{Min/+}$ mice (195).

In conclusion, despite its obvious tumor promoting roles, PLK1 inhibition in cancer has to be carefully evaluated as it can also compromise the aforementioned functions of PLK1 in spindle assembly and chromosomal stability, leading to increased chromosomal abnormalities and tumorigenesis.

4.2 PLK1 in other disorders

Although PLK1 undoubtedly holds the spotlight in cancer research, it has also been implicated in certain immune-mediated disorders. For example, alloreactive T cells upregulate PLK1 expression in Graft versus Host Disease, an adverse immunological phenomenon where donor T cells attack and destroy recipient's tissue. Conversely, PLK1 inhibition selectively prevented activation of alloreactive T cells and induced apoptosis and cell cycle arrest in already activated alloreactive T cells but not in memory T cells, making

PLK1 an attractive target in Graft versus Host Disease (13). Similarly, SIV-infected $CD4^+$ T cells in Rhesus macaques had augmented PLK1 levels, which led to deregulation of antigen presenting cell activation and to M phase defects (196). Additionally, a recent study revealed that $CD4^+$ T cells infected for the first time with HIV or latently infected $CD4^+$ T cells exhibit a PI3K- and Aurora A-dependent increase in PLK1 protein levels (197). It was found that HIV enhances the sumoylation of PLK1, leading to its nuclear translocation and stabilization, while PLK1 facilitates HIV survival in infected $CD4^+$ T cells. Consequently, PLK1 inhibition or knock down promotes cell death of HIV-infected cells offering a possible alternative approach to reduce latent HIV reservoirs in $CD4^+$ T cells (197). Upregulation of PLK1 in hepatic stellate cells (HSCs) has been linked with liver fibrosis (14). PLK1 overexpression in HSCs was shown to result in β-catenin accumulation and upregulation of the oncogenes MYC and Cyclin D1, whereas PLK1 inhibition abrogated the activation of HSC *in vivo* and enabled apoptosis of HSCs in liver fibrosis (14).

Finally, PLK1 has also been shown to contribute to neuronal cell death and is being studied in the context of Alzheimer's (16) and Huntington's disease (15,198), although the exact role of PLK1 in these disorders still awaits further studies.

5. Therapeutic targeting of PLK1

As discussed above, PLK1 expression is elevated in a wide range of tumors correlating with poor prognosis. PLK1 is a master regulator of cell cycle progression, but also plays a role in DNA damage response and apoptosis. As a result, PLK1 also contributes to cancer cell resistance to several chemotherapeutic agents and PLK1 inhibition enhances the sensitivity of tumors to conventional chemotherapy or radiotherapy in preclinical and clinical studies

(reviewed in (3)). An elegant study by Raab et al. showed that while primary cells are only weakly dependent on PLK1, cancer cells of different origins are “PLK1 addicted”, which in theory allows to develop tumor cell-specific PLK1 inhibitors (199). Consequently, PLK1 inhibition has been an appealing idea for both academia and pharmaceutical industry, who have made extensive efforts to develop small molecule PLK1 inhibitors (reviewed in (4,200)). Unfortunately, although several PLK1 inhibitors entered phase I and II clinical studies for patients with various cancers, in most cases they failed to achieve a satisfactory therapeutic effect due to dose-limiting toxicities (a brief update of clinical studies with PLK1 inhibitors is given in Table 3). Nevertheless, the search for strategies to limit PLK1 activity in cancer cells continues.

Small compound PKL1 inhibitors can be broadly categorized into ATP-analogues that target the PLK1 kinase domain and inhibit its catalytic activity, and compounds targeting the PBD. One of the first ATP-analogue PLK1 inhibitors that was developed is BI2536, which is capable of inducing mitotic arrest and apoptosis in human cells and promoting tumor cell death (201). Furthermore, BI2536 is a dual specificity inhibitor, as it was also shown to efficiently inhibit Bromodomain-containing protein 4 (BRD4), a transcriptional and epigenetic regulator required for tumor growth and survival and for which several other pharmaceutical drugs are currently being evaluated in clinical trials (202). BI2536 showed high efficacy and good tolerability in human xenograft models, eventually reaching phase I/II clinical trials for solid tumors, but its clinical development was discontinued due to modest response rate in patients with pancreatic cancer, NSCLC, AML, melanoma, head and neck cancer, soft tissue sarcoma, breast and ovarian cancer (201,203–206). However, BI2536 is still extensively used in research and was proven to repress synergistically tumor growth when combined with

other signaling inhibitors in many tumors, including triple negative breast cancer (TNBC) (167), B cell lymphomas (186), neuroblastoma (185) and glioblastoma (189).

Another very well-known PLK1 inhibitor, Volasertib (BI6727), has reached phase III clinical trials for AML. It is more potent than BI2536 and was shown to increase the efficiency of chemotherapeutics to suppress tumor growth in preclinical studies in several cancer cell lines and xenograft models, including AML (207), cervical cancer (208), thyroid cancer (209), melanoma (210) and hepatoblastoma (211). In 2013 Volasertib was granted the U.S. Food and Drug Administration (FDA) Breakthrough Therapy status for combined anticancer treatment together with cytarabine in AML. Combined therapy in AML patients aged 65 years and above improved rate response compared to monotherapy with cytarabine (31% vs 13,3 %) and improved overall survival (212). Volasertib in combination with low-dose cytarabine is still running in a phase III clinical trial for AML patients aged 65 years and above and is expected to finish in June 2021 (213). In addition to myeloid cells, treatment with Volasertib alone significantly inhibited cell proliferation in NSCLC cell lines, which was more pronounced in wild type p53 NSCLC cell lines compared to cell lines that had lost p53 function (214). However, Volasertib showed only moderate efficiency accompanied by toxicity in several clinical trials in patients with advanced or metastatic NSCLC (215), as well as in patients with platinum-resistant or refractory ovarian cancer (216) and patients suffering from advanced or metastatic urothelial cancer (217).

Most recently, two third generation highly specific PLK1 inhibitors (ATP antagonists) Onvansertib (PCM-075) and GSK461364 have been developed. Either alone or in combination with taxane drugs, both inhibitors induce apoptotic cell death in several cancer cell lines and promote tumor growth inhibition in xenograft models (218–221). In 2017, FDA

granted an orphan drug designation to Onvansertib for the treatment of AML patients. A phase Ib clinical trial with Onvansertib in combination with low dose cytarabine or decitabine showed well-tolerated responses related to myelosuppression and reduced circulating tumor DNA, which translated to clinical response, and will be further investigated in phase II clinical trials (NCT03303339) (222). Onvansertib is the only PLK1 inhibitor currently in clinical trials for solid tumors, and its safety profile was characterized in a Phase I dose escalation study in advanced and metastatic solid tumors. In 2020, FDA granted Fast Track designation to Onvansertib, for the second-line treatment of patients with KRAS-mutated metastatic colorectal cancer. Phase Ib using combined Onvansertib with FOLFIRI (a drug mix containing the chemotherapeutic drugs irinotecan, leucovorin, 5-fluorouracil), and bevacizumab as a second line treatment of metastatic colorectal cancer with a KRAS mutation is currently underway (NCT03829410) (223). Finally, a phase II study of Onvansertib in combination with abiraterone (an androgen synthesis inhibitor) in patients with metastatic castration-resistant prostate cancer demonstrated to be safe and showed some preliminary efficacy data (NCT03414034) (224). Onvansertib also inhibits neurogenic, adrenergic, and endothelin-1- and ATP-induced contractions of human prostate smooth muscle, which can lead to lower urinary tract symptoms and benign prostatic hyperplasia (225).

TAK-960 is a pyrimidodiazepinone analog developed in 2012, which inhibits PLK1 by targeting its ATP binding domain (226). It was proven to be potent and selective towards PLK1 and caused mitotic arrest in cancer cell lines and inhibited their proliferation regardless of the mutation status of p53 or KRAS (227). In addition, TAK-960 showed antitumor activities in several xenograft models including colorectal cancer and had a pharmacodynamics response in a paclitaxel-resistant leukemia mouse model (227). TAK-960

entered phase I evaluation in adult patients with advanced non-hematologic malignancies. However, this study was terminated due to lack of treatment efficacy and due to high frequency of serious diverse adverse effects including hematological and gastrointestinal side effects. Another selective ATP-competitive PLK1 inhibitor NMS-1286937 (NMS-P937) causes mitotic arrest and apoptosis in a variety of cancer cell lines (228), and combined treatment of NMS-1286937 with conventional chemotherapeutics leads to tumor regression in HT29 human colon adenocarcinoma xenografts (228). Furthermore, NMS-1286937 had a potent therapeutic effect in AML mouse models (229,230), increasing the survival rate of the treated groups. NMS-1286937 has reached phase I clinical trials for patients with advanced or metastatic solid tumors (231,232).

The first PLK1 inhibitors targeting the PBD were thymoquinone and its synthetic derivative Poloxin developed by Reindl et al. in 2008 (233). These inhibitors interrupt the proper localization of PLK1 and led to chromosome misalignments and apoptosis in HeLa cancer cells (233). Furthermore, Poloxin significantly suppresses tumor growth of cancer cell lines in xenograft mouse models, by repressing cell proliferation and by triggering apoptosis in tumor tissues (234). The major drawback of these compounds is, however, their toxicity, as high concentrations are required for an efficient anti-cancer effect. Moreover, it was recently shown that many of the PBD-blocking compounds are non-specific alkylating agents and might target multiple cellular proteins (235). Hence, the mitotic arrest caused by Poloxin and thymoquinone might rather reflect the inhibition of additional mitotic enzymes.

Rigosertib, one of the first developed PLK1 inhibitors (ON0910), is another non- ATP competitive small molecule PLK1 inhibitor (236). Rigosertib treatment leads to spindle abnormalities, induces apoptosis in HeLa cells and inhibits tumor growth in prostate, breast

and cervical cancer xenograft mouse models (236). However, Rigosertib is not specific to PLK1 and also targets PI3K (236–238). It has also been reported that Rigosertib kills cancer cells by directly binding and destabilizing microtubules, although the latter mechanism is a matter of debate (239–241). Rigosertib was also suggested to act as a RAS mimetic and to directly or indirectly inhibit RAS/RAF/MEK/ERK signaling (238,242). Rigosertib was tested in phase III clinical trials for patients with high risk of myelodysplastic syndromes after failure of hypomethylating agents, but did not show significant improvement compared to the standard of care (243). Furthermore, there were adverse effects linked to Rigosertib treatment, including anemia, thrombocytopenia and neutropenia. Combined treatment of Rigosertib with gemcitabine, a PI3K inhibitor, in patients with metastatic pancreatic cancer did not provide an added value in terms of overall survival or treatment response, compared to monotherapy with gemcitabine (244). A phase I study of Rigosertib on patients with relapsed B cell malignancies showed minimal toxicity, but no clinical significance was shown for Rigosertib as monotherapy (245).

Taken together, there are several small molecule PLK1 inhibitors in research and in early clinical development stages. Nevertheless, most of these promising molecules targeting PLK1 have been rather unsuccessful in clinical trials mostly due to a low therapeutic response and toxicity leading to serious adverse effects. PLK1 inhibitors that are currently in clinical trials are Volasertib (Phase III) for AML patients (NCT01721876) (213), Onvansertib (Phase II) for patients with colorectal cancer harboring a KRAS mutation (NCT03829410) (223), metastatic prostate cancer (NCT03414034) (224), ormetastatic pancreatic ductal adenocarcinoma (NCT04752696) (246), and Rigosertib (Phase III) for subsets of patients with very high-risk myelodysplastic syndrome (NCT02562443) (247) (Table 3). The challenges and limitations regarding the clinical implementations of PLK1 inhibitors rely on several reasons.

One of the most important obstacles is that resistance against an ATP-competitive PLK1 inhibitor can develop due to mutations in the kinase domain of PLK1. For instance, a single point mutation (Cys67Val) in the kinase domain of PLK1 leads to resistance to BI2536 (248). In addition, PLK1 inhibitors also target PLK2 and PLK3 (although to a lesser extent) due to the high homology in the kinase domain between these three PLK members. This is a matter of concern, as there is growing evidence that both PLK2 and PLK3 have tumor-suppressing properties. This is supported by the observation that PLK3-deficient mice have significantly increased tumor incidence in the kidney, lungs, uterus and liver (249). Also, PLK2 is a direct transcriptional target of the tumor suppressor p53 and the p53-dependent activation of PLK2 prevents mitotic catastrophe following spindle damage (250). Taken the above studies into consideration, ideally a PLK1 inhibitor should not compromise the activity of other PLK family members. Another challenge that ATP analogue-type PLK1 inhibitors present with is that most of them require a high concentration (sometimes micromolar) in order to efficiently achieve desired therapeutic effects, while these high concentrations can lead to toxicity. Moreover, there is an off-target effect to other kinases, interfering with normal processes, which often results in toxicity including neutropenia and myelosuppression, adverse effects which are also caused by microtubule-targeting agents. As PLK1 is mainly expressed during the G2/M phase, only a small fraction of cells is vulnerable to PLK1 inhibitors, making it challenging to use PLK1 inhibitors against slowly growing tumors. In spite of the fact that PLK1 inhibitors offer an exciting and promising therapeutic strategy to defeat cancer, due to the above discussed limitations they are still not considered a “magic bullet”. However, the combined use of PLK1 inhibitors with conventional chemotherapeutics has been promising, as there is a synergistic effect of PLK1 with microtubule-binding agents, making combined therapy much more efficient than

monotherapy with PLK1 inhibitors. In order to maximize this synergistic effect, deep understanding of the PLK1 regulatory network and its interaction with other signaling pathways is necessary.

6. Conclusions and future perspectives

PLK1 is best known as a major regulator of mitosis, as it controls timely mitotic progression, centrosome maturation and separation, spindle assembly formation, chromosome alignment and segregation, as well as cytokinesis and abscission. Deregulation of PLK1 can therefore have many detrimental implications, which is best illustrated by its well documented involvement in carcinogenesis. The high expression levels of PLK1 in several tumor cells may cause chromosome instability, lead to deregulation of many oncogenic pathways including *TP53* and *MYC*, and promote EMT. Accordingly, PLK1 overexpression is often associated with poor prognosis and tumor aggressiveness, which has driven the development of PLK1 inhibitors as new anti-cancer drugs. However, only very few of them showed any promising therapeutic effects in clinical trials, which may stem from toxicity, which is partially due to prohibitively high dosages used, pleiotropic functions of PLK1 in mitotic cells, as well as off-target effects. Use of lower doses of PLK1 inhibitors in combination with conventional chemotherapeutic agents may still be a way to go and is an approach that is currently investigated. The complexity associated with PLK1 targeting in cancer is further increased by the fact that PLK1 inhibition may also accelerate mitotic exit of cells harboring chromosome misalignments, contributing to oncogenesis. Therefore, PLK1 may act as a double-edged sword, being able to promote or suppress tumor development, complicating the therapeutic use of PLK1 inhibitors. Future studies should therefore focus

on a better understanding of the molecular mechanisms that mediate and regulate PLK1 activity in different normal cells and cancer cells, including the existence of other genetic vulnerabilities or signaling pathways that may act in parallel or even intersect with PLK1 signaling. Moreover, new insights in the diverse cellular functions of PLK1 beyond cell cycle regulation may become equally important. In this context, initial evidence pointing towards a regulatory role for PLK1 in NF-κB signaling indicates an interesting path for future work as NF-κB signaling plays a major role in tumorigenesis (251). Furthermore, regulation of NF-κB signaling as well as IRF3 signaling by PLK1 in normal cells may also point towards a largely unexplored role of PLK1 in immunity and inflammation, which may have far reaching implications in immune-mediated disease. The proposed involvement of PLK1 in serious neurological disorders, such as Alzheimer's and Huntington's disease, suggests that PLK1 can regulate other unexplored signaling pathways (e.g. the Hippo pathway) in neurons and other cell types. There is a high chance that PLK1 is implicated in many more cellular processes, where deregulation of either PLK1 itself or of its downstream targets could lead to interesting phenotypes and the onset of disease. Therefore, it will be of utmost importance to broaden our knowledge on the role of PLK1 signaling beyond mitosis, which may eventually reveal novel opportunities for therapeutic PLK1 targeting using small compound inhibitors.

Competing Interests

"The authors have declared that no competing interests exist."

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Figure legends

Figure 1: Domain structure of the five mammalian PLKs.

The N-terminal catalytic kinase domain is depicted in red, and a C-terminal Polo-box binding region is depicted in blue. PLK4 also contains a Cryptic Polo-box domain region (depicted in dark blue). PLK5 has a non-functional truncated kinase domain.

Figure 2: Role of PLK1 in mitosis.

Schematic diagram of the role of PLK1 in the different phases of mitosis. Note that for reasons of simplicity not all PLK1 substrates are depicted. **A.** PLK1 in centrosome maturation. PLK1 together with Aurora A kinase are cooperatively recruited to CEP-192, where they create docking sites for γ-tubulin ring complexes (γ-TURC). The interaction of PLK1 with other centrosomal proteins, such as Gravin and Cenexin, further facilitates localization of PLK1 to centrosomes, promoting γ-tubulin recruitment. **B.** PLK1 in mitotic entry. PLK1 activation by combined action of Bora and Aurora A kinase leads to the degradation of CDK1 inhibitor WEE-1 and inhibits activity of MYT-1, while simultaneously phosphorylating Cyclin B and promoting its nuclear export. In parallel, PLK1 also phosphorylates CDC25C, a phosphatase responsible for the activation of the Cyclin B1/CDK complex. **C.** PLK1 in kinetochore-microtubule attachment. PLK1 gets recruited to kinetochores by several proteins including PBIP1, BUB1 and BUBR1, where it phosphorylates KNL-1 and MPS1, promoting mitotic checkpoint complex (MCC) formation and blocking mitotic exit. When chromosomes are correctly aligned, PP2A-B56 phosphatase removes PLK1 from kinetochores, initiating transition to anaphase. **D.** PLK1 in mitotic exit. In late anaphase PLK1 phosphorylates EMI-1 leading to its proteasomal degradation to enable activation of the APC/C complex. **E.** PLK1 in cytokinesis. PLK1 interacts with and

phosphorylates MKLP1 kinesin in the midzone, which is required for the spatial restriction of PLK1 during anaphase and telophase. Additionally, PLK1 binds and phosphorylates HsCYK-4, creating a docking site for ECT2, which is instrumental for the contractile ring assembly.

Figure 3: Role of PLK1 in DNA replication and DNA Damage Repair (DDR)

A. PLK1 in DNA replication. DNA replication starts with the orderly assembly of the pre-replicative complex (pre-RC) at the origins of replication consisting of several proteins. In stress conditions, PLK1 interacts with and phosphorylates several components of the pre-RC including ORC2, MCM2-7 and HBO1, promoting DNA replication. **B.** PLK1 in DDR. DNA damage induces the activation of the ATM/ATR and CHK1/2 kinases cascade, which ultimately leads to CDC25 degradation and mitotic block. PLK1 activation is inhibited in an ATR-dependent way by interfering with Aurora A interaction with Bora-PLK1 and Bora degradation. Also, the levels of PLK1 methylated at Lys209 increase, preventing its activation by Aurora A. Conversely, during checkpoint recovery PLK1 phosphorylates Claspin that is required for ATR activation inducing its proteasomal degradation and cell cycle progression.

Figure 4: Regulation of NF-κB and RIG-I signaling by PLK1

A. PLK1 in NF-κB signaling. Activation of interleukin-1 receptor (IL-1R) or Toll-like receptors (TLR) on the cell surface induces IKK complex (composed of IKKα, IKKβ, and NEMO) formation and IKK-mediated phosphorylation of the NF-κB inhibitory protein IκBα, inducing its proteasomal degradation and freeing NF-κB dimers (p50/p65) to translocate to the nucleus and initiate expression of specific target genes. PLK1 phosphorylates IKKβ preventing its recruitment into the IKK complex, ultimately inhibiting NF-κB activation. In addition, PLK1 interacts with TRAF-associated NF-κB activator (TANK), leading to

recruitment of PLK1 to the IKK complex where it reduces the ubiquitination of NEMO, further contributing to reduced NF-κB activation. **B.** PLK1 in RIG-I/MAVS signaling. Viral dsRNA or ssRNA is recognized by RIG-I, resulting in activation of the mitochondria-bound MAVS. This leads to recruitment of TRAF3 and ultimately to TBK1/IKKε-mediated phosphorylation of IRF3 transcription factors, enabling their translocation to the nucleus and induction of type I interferons (IFNs). PLK1 interacts with the N-terminal end of MAVS in a phospho-dependent manner, as well as with the C-terminal domain of MAVS independently of MAVS phosphorylation. The latter interaction prevents the recruitment of TRAF3 eventually leading to the inhibition of IFN induction.

Table 1. Overview of PLK1 substrates and biological functions related to cell cycle events

Substrate	P-Site	Result	Biological function	Ref.
Centrosome life cycle				
CEP192	Thr44 Ser995	PLK1 localization	Centrosome maturation	(60)
Cenexin	Ser796	PLK1 localization	Centrosome maturation, recruitment of γ-TURC	(64)
HDAC6	Docking only Site ND	Sub activation	Cilia deacetylation and resorption	(74)
DVL-2	Docking only Site ND	Sub-PLK1 complex	Cilia disassembly HEF-1 stability regulation => HDAC6 activation	(76)
DZIP1	Ser210	Sub dissociation from a multiprotein complex	Ciliary disassembly	(73)
MST-2	Ser15, Ser18, Ser316	Prevents PP1γ binding to Sub	Regulation of centrosome separation by NEK2A	(65)
PCNT	Ser2259	Separase-mediated cleavage	Centriole separation	(71)
	Ser1235 Ser1241	Sub recruitment to centrosomes	Centrosome maturation at onset of mitosis	(68)
DNA replication				

TopoIIα	Ser1337 Ser1524	Sub activation	DNA replication, DNA unwinding	(113)
FOR20	ND	PLK1 sequestration	Initiation of DNA replication	(112)
HBO1	Ser57	Sub recruitment to pre-RC	DNA replication licensing, pre-RC assembly	(107)
ORC2	Ser188	Recruits MCM components	Pre-RC maintenance and DNA replication under stress	(105)
MCM2	Docking only Site ND	PLK1 localization	DNA replication under stress	(106)
Mitotic entry				
WEE-1	Ser53	Sub degradation	CDK1 activation	(37)
Cyclin B1	Ser133 Ser147	Sub nuclear localization	CDK1 activation	(40,81)
CDC25C	Ser198	Sub activation and nuclear translocation	CDK1 activation	(39)
MYT-1	Ser426 Thr495	Sub inactivation	CDK1 activation	(41,80)
CYLD	ND	ND	Interaction with PLK1 is required for mitosis entry	(82)
Bora	Docking only Site ND	PLK1 activation Sub degradation	Entry to mitosis	(46,47)
14-3-3γ	Ser99 on PLK1	PLK1 activation	Cell cycle progression to anaphase	(49)
Mitosis				
PBIP1	Thr78	PLK1 localization to kinetochores	Kinetochore-microtubule attachment	(35)
NUDC	Ser274 Ser326	PLK1 localization to kinetochores	Chromosome alignment to the metaphasic plate	(83)
INCEP	ND	PLK1 localization to kinetochores	INCEP/PLK1/Aurora B => metaphase to anaphase	(84,85)
MPS-1	Thr33 Ser37 Ser363	Sub activation	Enhanced MCC formation, SAC maintenance	(89)
KNL-1	Thr875	Creates docking sites	Enhanced MCC formation, SAC maintenance	
BUB1	Thr609 docking site	Targets PLK1 at kinetochores	Supports MCC function	(38)
BUBR1	Ser676	Targets PLK1 to kinetochores/centrosomes	Stable kinetochore-microtubule interactions	
NuMA	site ND	Sub localization	Regulates spindle orientation	(92)
CEP76	Docking site Site ND	PLK1 sequestration to centrosome	Correct spindle orientation	(93)
BRCA2	Thr207	BRCA2-pBUBR1-PLK1-PP2A complex	Stable kinetochore-microtubule interactions	(8)

RIPK1	Docking, site ND	PLK1 recruitment to the ripoptosome, cleavage	Chromosome stability	(7)
EMI-1	Ser145 Ser149	Sub degradation	APC/C complex activation, entry to anaphase	(91)
Cytokinesis and abscission				
HsCYK-4	Ser149, 157, 164, 170, 214, 260	Recruitment of ECT2	Stimulates contractile ring assembly	(36)
MKLP2	Ser528	Localizes PLK1 to the central spindle	Spatial restriction of PLK1	(99)
PRC1	Docking on Ser601/ Thr602	PLK1 recruitment to kinetochores	Centralspindlin release from the midzone to the equatorial cortex	(100)
CEP55	Ser436	Regulates CEP55 recruitment	Prevents abscission	(103)

Sub: substrate; ND: not determined

Table 2. Overview of PLK1 substrates and functions related to cancer

Substrate	P-Site	Result	Ref
Inhibition of tumor suppressors			
GTSE1	Ser435	GTSE1 nuclear localisation => p53 nuclear export and degradation	(151)
Topors	Ser718	↗ p53 ubiquitination and degradation	(175)
NUMB	Ser265	NUMB degradation => p53 degradation	(12,176)
MDM2	Ser260	p53 degradation	(143)
p53	DNA binding domain	p53 inhibition	(152)
PTEN	Ser385	↘ PTEN activity and nuclear localization => PI3K/AKT activation, cell proliferation	(177)
FOXO1	Ser75	FOXO1 inhibition => apoptosis inhibition	(184)
REST	Ser1030	REST degradation	(167)
SUZ12	Ser539 Ser546	SUZ12 degradation Cellular reprogramming and transformation	(182)
ZNF198	Ser305	ZNF198 degradation	
Activation of tumor promotors			

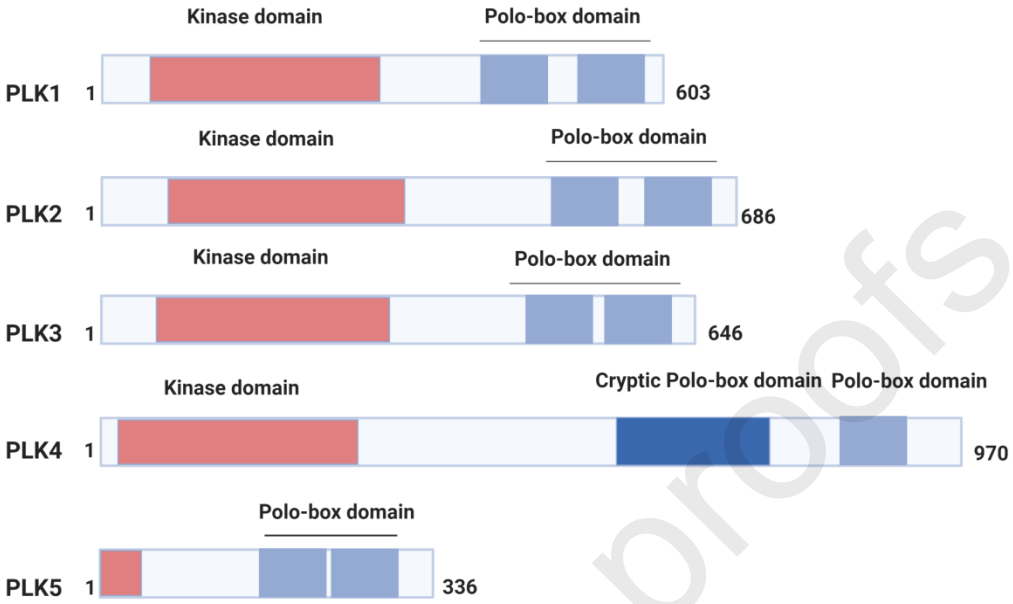
FBW7	Ser58 Thr284	FBW7 degradation => ↗ N-MYC, Cyclin E, MCL-1	(185)
AKT/GSK3β activation	PTEN at Ser385	PTEN inactivation => MYC stabilization	(178)
KLF4	Ser234	Tumor promotor KLF4 stabilization	(188)
STAT3	ND	Reciprocal regulation of PLK1 and STAT3 via β-catenin => ↘ apoptosis	(189,190)
Epithelial Mesenchymal Transition			
cRAF	Ser338, 339, 621	MEK/ERK activation => ZEB1/2 activation	(158)
AKT	Ser473	↗ stability of SNAIL	(159,162)
Metabolism			
PI3K/AKT pathway activation	PTEN at Ser385	Aerobic glycolysis	(178)
G6PD	Thr406, 466	Pentose phosphate pathway activation	(191)

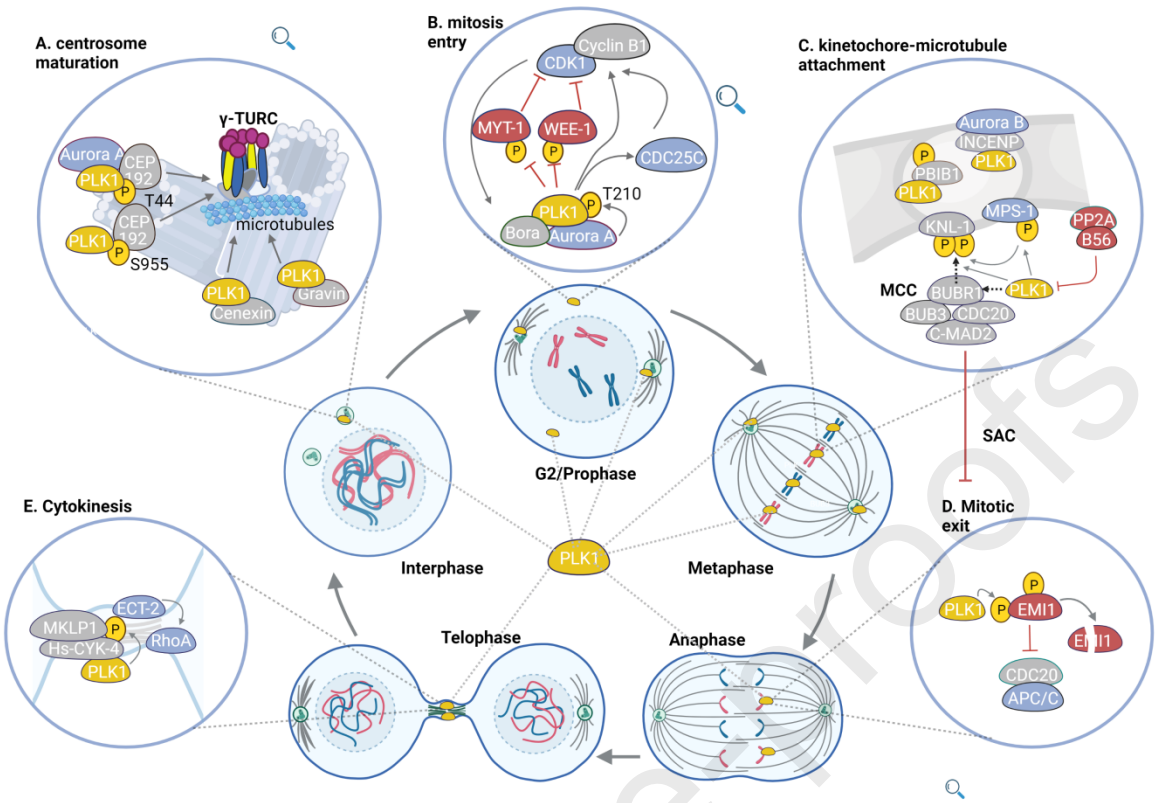
Table 3. PLK1 inhibitors in clinical trials*

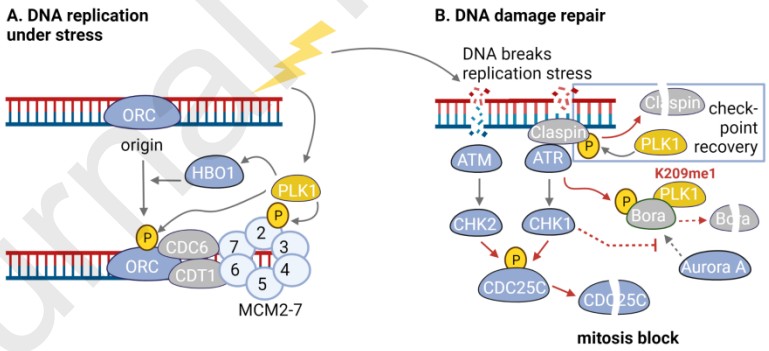
Compound	Target	Clinical Trial Phase	Clinical trial status	Tested against	Outcome	NCT number
BI2536	ATP-binding	I/II	Completed/ Terminated	Solid tumors	Modest response rate	11 studies in total
Volasertib BI6727**	ATP-binding	III	Completed, June 2021	AML	No results available	01721876
Onvansertib PCM075	ATP-binding	II	Active, recruiting Estimated to be completed in May 2022	Metastatic colorectal cancer with a KRAS mutation	Not available yet	03829410
		II	Active, recruiting Estimated to be completed in May 2022	Metastatic castration-resistant prostate cancer	Not available yet	03414034
		II	Active, recruiting Estimated to be completed in March	Metastatic pancreatic ductal	Not available yet	04752696

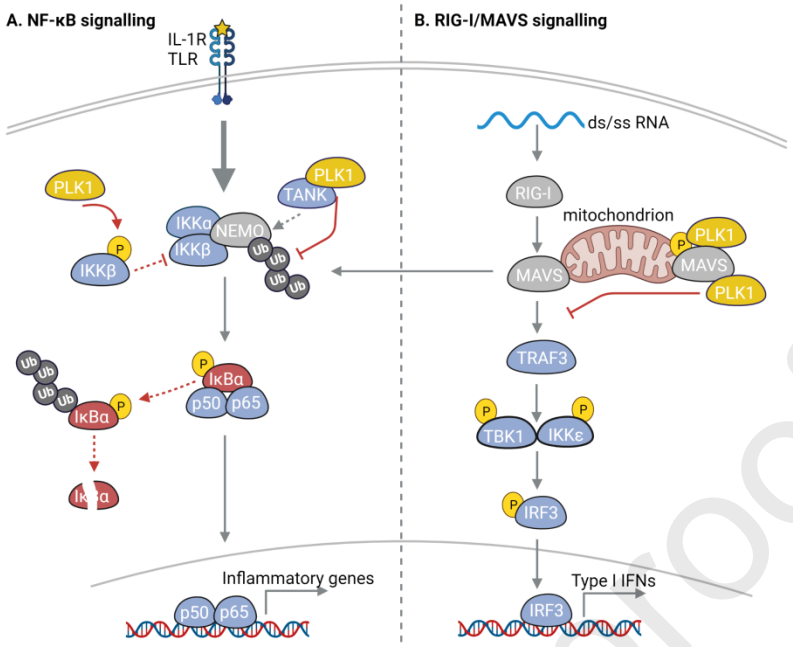
			2024	adenocarcinoma		
GSK461364	ATP-binding	I	Completed, 2009	Non-Hodgkin's lymphoma	Toxicity (20% thrombotic emboli), 15% efficacy	00536835
TAK-960	ATP-binding	I	Terminated, 2013	Advanced non-haematological malignancies	Discontinued due to business reasons	01179399
NMS-1286937	ATP-binding	I	Completed, 2011	Advanced or metastatic solid tumors	Disease stabilization	01014429
Rigosertib	Non-ATP competitive	III	Completed, 2015	Untreated metastatic pancreatic cancer	No improvement in survival or response, neutropenia	01360853
		III	Completed, 2017	Myelodysplastic syndrome	Safety review ↑gastrointestinal ↑ urinary adverse effects	01928537
		III	Active, not recruiting, estimated to be completed in December 2021	Myelodysplastic syndrome	Not available yet	02562443

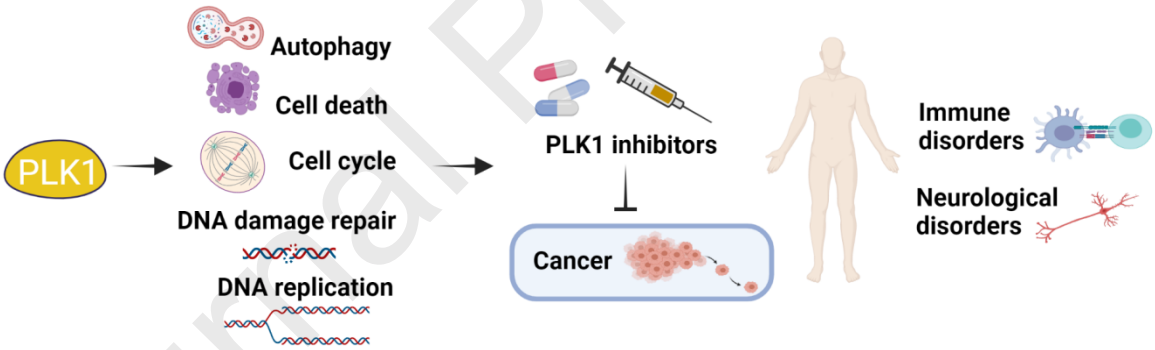
*Data source: ClinicalTrials.gov; ** a representative study is shown; + 26 other finished studies not mentioned here.











Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement

Styliani Iliaki: Writing- original draft, Visualization. **Rudi Beyaert**: Writing – Review and Editing, Supervision, Funding Acquisition. **Inna S. Afonina**: Writing – Review and Editing, Visualization, Supervision, Funding Acquisition.